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Applicant:	Xiaoyang Qi	: Paper No:	
Serial No.	10/801,517	: Group Art Unit:	1643
Filed:	March 16, 2004	: Examiner:	Sang, Hong
For:	SAPOSIN C-DOPS: A NOVEL ANTI-TUMOR AGENT		

Confirmation No. 4062

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This declaration under 37 CFR Sec. 1.132 is supportive of the Amendment and Response filed herewith. I, Xiaoyang Qi, declare and say:

1. That I am a citizen of the United States and that I am the inventor of the above-referenced patent application; that I am employed by Cincinnati Children's Medical Center as an Assistant Professor, and I was and still am, engaged in a research program in the field of biochemistry and molecular biology of saposins.

2. That I am familiar with the above-identified patent application Ser. No. 10/801,517, that I have reviewed the August 28, 2007 Office Action in the above captioned case; and that I am familiar with the following reference: Vaccaro et al. (FEBS 1993, 336(1): 159-162) in view of the teachings of O'Brien et al. (W09503821A1), as evidenced by Vaccaro et al. (FEBS, 1994, 349: 181-186, IDS).

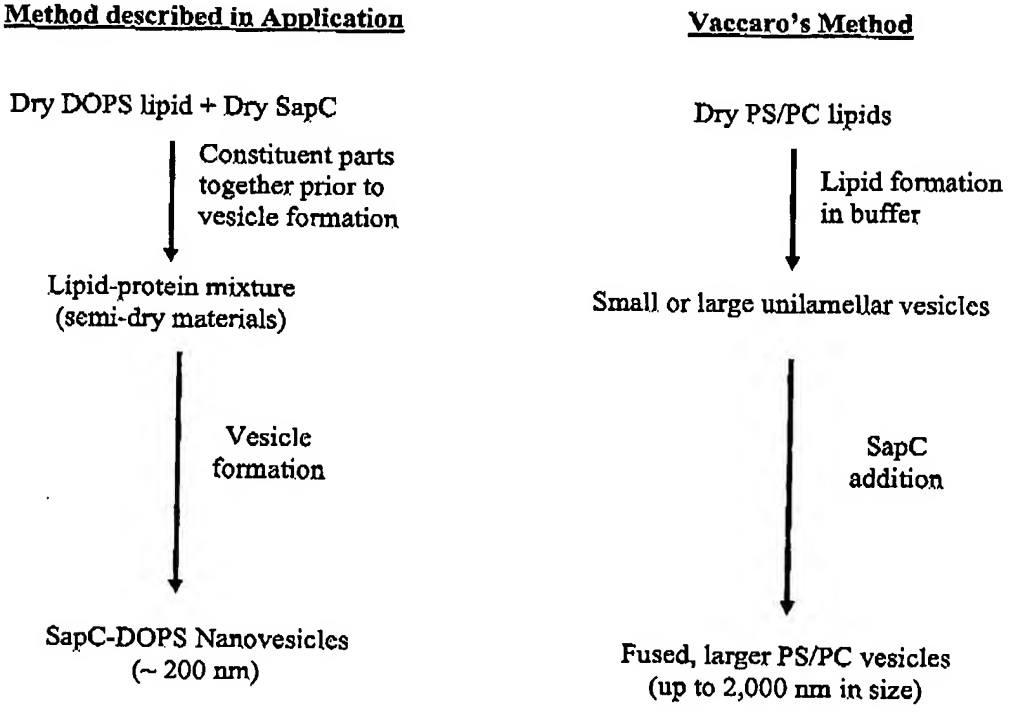
3. That I contend that the in the rejection the Examiner states that the teachings of Vaccaro and O'Brien show forming liposomal vesicles and then adding Saposin C (SapC) to the formulation, resulting in a surface interaction of the protein with the vesicles. A lipid/saposin vesicle formed by this method will not function the same and will not exhibit anti-tumor activity as with the vesicles of the present invention.

4. That the present invention is not a case of simply a composition comprising a mixture of lipid nanovesicles and polypeptide but is a composition comprising a SapC-DOPS nanovesicle complex. That the composition of the present invention comprises a SapC-DOPS

nanovesicle complex and that it would be clear to one skilled in the art from the description of the present invention within the specification, especially as described in Example 2, that the composition comprises a SapC-DOPS nanovesicle complex and not a mixture of nanovesicles and SapC suspended in a carrier.

5. That the methods and compositions described in the Vaccaro reference [FEBS 1993, 336(1): 159-162] are not the same as those described in the present application. The Vaccaro reference actually shows that addition of SapC to PS-only liposomes alters the size and morphology of the liposomes. The liposomes, as shown in the electron micrographs of Fig. 6 in a subsequent publication [Vaccaro *et al.* FEBS Letters 349(1994)181-184], are a much larger liposome that is not a nanovescicle having SapC integrated within its structure.

The nanovesicles, made from the materials and methods of the present invention, are much smaller liposomes (generally about 200 nm or smaller) that exhibit anti-tumor activity. The liposomes of the Vaccaro reference are created first with SapC added after formation resulting in fused, larger sized PS/PC phospholipid vesicles that are up to 2000 nm in size or larger. This creates a different product where the polypeptide merely adheres to the surface of the large liposomes and renders liposome that fail to show significant anti-tumor activity.



6. That I disagree with the Examiner's position and maintain that one of ordinary skill in the art would understand how to make and use the present invention and that the specification does provide proper guidance on how to make the class of polypeptide that has the claimed function. It is well within the capability of one of ordinary skill in the art, through routine laboratory procedures, to ascertain whether or not a given polypeptide retains plasma-membrane affinity and whether or not a given nanovesicle exhibits anti-tumor activity.

7. That it is well known in the art that the proteins of the invention may be altered in various ways including the amino acid substitutions, deletions, truncations, and insertions. Moreover, applicants have provided sufficient detail of particular patentable embodiments and a person skilled in the art can easily ascertain the sequences that fall within the scope of the present claims given that whether or not a polypeptide falls within the scope of the present claims.

8. That I have performed the following studies to show that the Saposin C polypeptide, when mixed with nanovesicles after the nanovesicles have been formed, does not exhibit the same effect as the SapC-DOPS nanovesicle complexes, formed by the processes of the present application. These results show that a co-treatment of Saposin C and the DOPS vesicles provide for no significant cancer cell killing (Table 1) and tumor targeting (Figure 1) effects.

Table 1.

Treatments of neuroblastoma (CHLA-20) cells with protein and/or lipid.

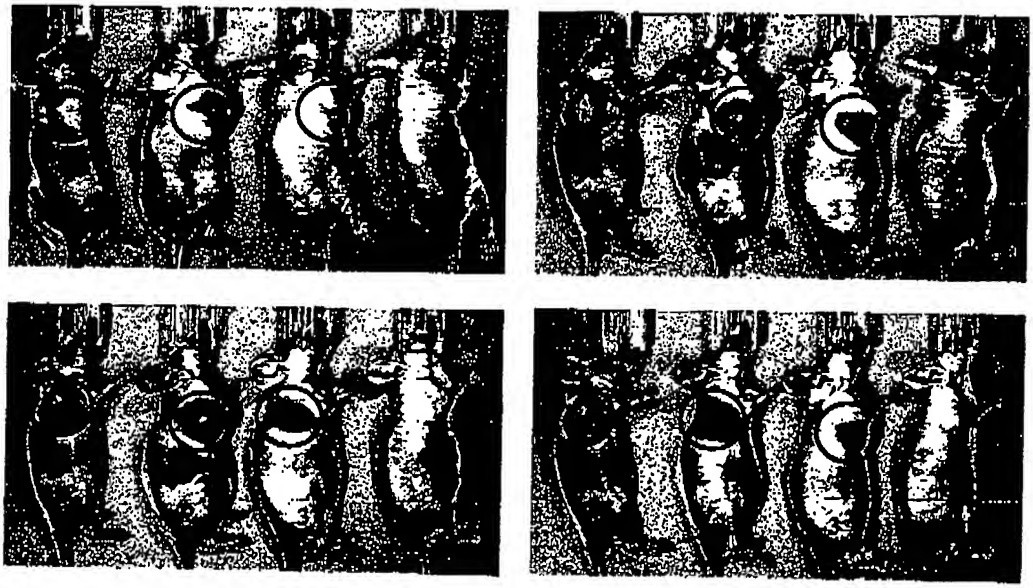
Human Cells	Cell Death (%)	
Cancer	**Untreated**	**SapC-DOPS**
Neuroblastomas: CHLA-20	13.8 ± 3.8	87.2 ± 0.7
Cancer	**Untreated**	**SapC and DOPS**
Neuroblastomas: CHLA-20	13.8 ± 3.8	20.6 ± 6.1
Cancer	**Untreated**	**DOPS**
Neuroblastomas: CHLA-20	13.8 ± 3.8	11.9 ± 3.4
Cancer	**Untreated**	**SapC**
Neuroblastomas: CHLA-20	13.8 ± 3.8	12.2 ± 1.6

Experimental conditions: Cells (4 x 10^4/100 µl/well) were cultured for 24 h prior to the treatment. MTT assay was carried out after cultured the treated cells for 3 days. Spectrophotometric data from quadruplication wells (in 96 well plates) were analyzed by ANOVA. The data were presented as the arithmetic mean ± SEM. Experiments performed at least twice. Protein = 100 µM; Lipid = 300 µM.

Treatments:
SapC-DOPS: nanovesicle complexes, significant killing effect.
SapC and DOPS: co-treatment of SapC and the DOPS vesicles, no significant killing effect.
DOPS: DOPS vesicles alone, no significant killing effect.
SapC: SapC alone, no significant killing effect.

Figure 1. Shows tumor-targeting of fluorescent labeled SapC-DOPS nanovesicles *in vivo*.

Xenografted neuroblastoma tumor-targeting of the CVM-labeled SapC-DOPS using the IVIS 200 live imaging system. Heterotopic neuroblastoma tumors (circled in mouse 1, 2, and 3) were generated by subcutaneous injection in the upper flank of nude mice. The samples were administrated into mice through tail vein injection. Time points were 0, 12, 24, 48 h post-treatment. CVM: CellVue™ Maroon (MTTI, Co.) fluorophore. IVIS Filters: Ex = 640 nm, Em = 700 nm. Mouse 1: the CVM-labeled SapC-DOPS nanovesicles, strong tumor-targeting effect; Mouse 2: co-administration of SapC and the CVM-labeled DOPS nanovesicles, no tumor-targeting effect; Mouse 3: the CMV-labeled DOPS nanovesicles, no tumor-targeting effect; Mouse 4: non-tumor bearing with PBS injection, no tumor-targeting effect.



9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Further declarant sayeth not.

Dr. Xiaoyang Qi

2/28/08

Date

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